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III. THE EFFECTS OF BARNYARD MANURE

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SUMMARY.

The conclusions which may be drawn from the experiments upon the effects of barnyard manure on bacteriological activities of field soils presented in this bulletin are as follows:

1. Applications of manure up to sixteen tons per acre increased the numbers of organisms in the soil as shown by the growth on modified synthetic and albumen agar. The ammonifying power of the soil as shown by tests with the casein-fresh soil, albumen-fresh soil, dried blood-fresh soil, and dried blood-air-dried soil methods, and the nitrifying power tested by the ammonium sulfate-fresh soil, and the ammonium sulfate-air-dried soil methods were likewise increased.

2. The greatest increases occurred between the check soil and that receiving eight tons per acre and between the soil receiving the eight tons and that to which twelve tons per acre were applied. In most cases only a very slight increase occurred in the soil on which sixteen tons were used over that where twelve tons were added.

3. Twenty tons of manure per acre caused a depression in numbers of bacteria, in ammonifying power, and in nitrifying power according to all the methods employed, the results being lower than those secured when twelve tons per acre were added.

4. Albumen agar permitted of the development of larger numbers of soil organisms than the modified synthetic agar and also permitted of a greater differentiation between the soils of the various plots.

5. There was a close relationship between the ammonifying power of the soils and the numbers of organisms in them accord-to the methods used in this work.

6. The casein-fresh soil method of testing the ammonifying power of the soil was the simplest, permitted of the greatest differentiation between different soils, and in general was the most satisfactory.

7. The ammonium sulfate-fresh soil method for testing the nitrifying power of the soil showed the greatest differences between the various soils and is recommended as the more rational method.

8. The nitrifying power and the ammonifying power of the soil according to the methods used proceeded in the same direction.

9. Applications of manure up to sixteen tons per acre increased the yield of corn from the plots in this series, the great-

est increases occurring between the check plot and that receiving eight tons per acre and between the latter and the plot to which twelve tons per acre were added.

A very slight further increase occurred when sixteen tons per acre were applied.

10. Twenty tons of manure per acre depressed the crop yield below that obtained when twelve tons per acre were added.

11. The results of the bacteriological tests and the crop yields coincide almost exactly. Further evidence is thus supplied that there is a close relationship between bacterial activities and the fertility or crop-producing power of soils.

12. The depression in crop yields and bacterial activities caused by twenty tons of manure per acre cannot be attributed to denitrification as tests by the Giltay solution method and the soil method give no evidence of losses of nitrogen. The depression must therefore be due to physiological or other causes.

THE EFFECTS OF BARNYARD MANURE

BY PERCY EDGAR BROWN

The use of farm manure to increase crop yields was common more than two thousand years ago, but the reasons for such increase remained a mystery until the brilliant researches of Liebig in the middle of the last century. He showed that certain chemical elements were essential for the growth of plants and that manure contained varying amounts of these elements. It was believed then, therefore, that the beneficial effect of manure was due to the addition of these chemical plant food constituents to the soil. Now it is commonly recognized that farm manure exerts a fourfold effect on soils; not only a chemical, but also a physical, a bacteriological and a physiological effect. Knowledge of the last is still somewhat hypothetical and is based on the supposed toxic influence of organic substances on crops. The physical effects of manure as, for example, in opening up tight soils and rendering light, open soils more retentive of moisture and plant food, are too well known to need any discussion, as are also the chemical effects which consist mainly in adding plant food to the soil.

The bacteriological effects of manure, however, have not been extensively studied, although receiving some attention now in connection with the investigations in soil bacteriology, and no definite principles governing the action of manure on bacterial processes in the soil have yet been evolved. This is due to two reasons. First, the methods employed to test bacterial activities in the soil have been so unsatisfactory and so constantly changing that the results obtained by their use can hardly be regarded as showing the actual extent and importance of soil bacterial processes or as bringing out the true differences in soils due to the effects of different treatments. In the second place, results of tests of soils kept under the artificial conditions of the greenhouse or laboratory should not be considered as more than indicative of what may be occurring under actual field conditions. As has been pointed out by various writers, the air, moisture, and general climatic conditions exert such a vital effect on the bacteria in soils that unless these conditions are natural, the results of bacterial tests can hardly be considered applicable to field soils.

The many difficulties encountered in examining field soils were pointed out in the first work in this series, and also the means by which some of them may be eliminated or at least so far overcome as to make them negligible.

USE OF MANURE INVOLVES MANY QUESTIONS.

Many questions are involved in the use of manure on soils and considerable research will be necessary before a definite understanding of the principles concerned and the effects produced can be reached. There must be chemical study of soil organic matter and the organic constituents of manure; likewise, there must be study of the effects of the various compounds in different manures on important groups of bacteria and on various crops. In short, the problem is very complicated, so complicated that it would be clearly unwarranted, if not absolutely ridiculous, to say that the effects of manure are entirely physiological. It is just as unwarranted to claim that the chemical or the physical effects are of prime importance. The bacteriological changes brought about by manure must be considered fully for they are very largely dependent on its chemical and physical effects and may, therefore, be regarded as indicative of the trend of these other effects. No claim can be made, however, that the bacteriological changes are more vital than the others, but merely that they are of more significance. Physical and chemical effects of manure on crop yields are not brought about directly by physical and chemical means but indirectly by definite influence on bacterial activities. Furthermore, it is not too great an assumption to say that the physiological effects of manure, if there be such, are probably not caused entirely by physiological action on the plants but indirectly by physiological action on soil organisms.

It is not the purpose of these experiments, however, to undertake to solve the complicated problem of the various effects of manuring. Considerable work under a great variety of conditions will be necessary before anything approaching a solution will be possible. This work was begun merely to throw some additional light on the bacteriological phase of the problem from the field standpoint and also to secure additional data regarding the relation between bacterial activities and actual crop yields. Earlier work on field soils and some results still unpublished indicate the existence of a definite relation between certain bacterial processes in the soil and the crops produced on them, but much more data is necessary for any definite conclusion. Also, as will be noted later, new methods for the bacteriological examination of soils recently devised are to be tested and further evidence of their value secured.

HISTORICAL.

All experiments dealing with the effects of manure on the bacteria in the soil have shown not only a considerable increase in the numbers of organisms but also in the activities of certain groups of organisms particularly important from the fertility standpoint. The increase in actual numbers of organisms is to be expected when it is remembered that manure contains an enormous number of bacteria, variously estimated at from 7,000,000 to 375,000,000 per gram. Furthermore, the materials present in manure which serve as bacterial food naturally encourage the multiplication of organisms to a large extent.

Among the investigators reporting increases in numbers of organisms present in soil upon application of manure may be mentioned Caron[1], Remy[2], Fabricius and Von Feilitzen[3], Engberding[4], and H. Fischer[5].

In a more recent work Temple[6] showed that an addition of ten tons of cow manure per acre to a soil greatly increased the number of bacteria in that soil and that this increase continued over a considerable period. He found also that sterilized manure caused a greater increase than unsterilized. Thus it would seem that in this particular case the chemical or physical composition of the manure carried more influence than the bacterial content. Probably the sterilized manure encouraged the growth of a particular group of organisms which developed on the medium employed in the counts and to such a large extent that greater numbers were obtained than was the case when the manure containing its millions of living organisms was used. The possibility that the sterilization of the manure in the first case changed the chemical character of the manure, as well as the possibility of some toxic effects of the unsterilized material on the soil organisms, must be considered.

On the other hand Hellstrom[7] found that on moor soils fertilized with sterilized and unsterilized manure, the largest yields were obtained with the unsterilized material. He concluded that this effect was due to the bacteria added in the manure. Unfortunately, Temple did not have the crop yield to compare with his bacteriological results and Hellstrom did not determine numbers and activities of bacteria, so that we must attribute the discrepancy between the two results to the chemical and bacterio-

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[1]Vortrag. geh. in. d. Wintervers.d. Zentralausschusses. d. Landw. gesellsch. Hanover., 26, XI. 1896 (ref. Jahresber. d. Garungsorg., 8, p. 212).
[2]Centbl. f. Bakt., Abt. II., Bd. 8, 1902, p. 734-762.
[3]Centbl. f. Bakt., Abt. II., Bd. 14, 1905, p. 165.
[4]Centbl. f. Bakt., Abt. II., Bd. 23, 1909, p. 601.
[5]Landw. Jahr., Bd. 38, 1909, p. 358.
[6]Bull. 95. Georgia Agr. Expt. Stat., 1911.
[7]K. Dandt. Akad. Handl. 33 (1899) p. 167-171.

logical differences in the soils and in the manure employed, which, of course, may be large.

Temple concluded also that additions of cow manure increased the ammonifying efficiency of most soils whether it was applied in a sterile or unsterile condition. The results of his ammonification studies, therefore, did not confirm the quantitative determinations. In the case of nitrification, however, while an increase in the nitrifying efficiency of most soils was shown, the greatest increase occurred when unsterilized manure was added. This greater effect of unsterilized manure was found to be due to the actual addition of nitrifying organisms. Niklewski[8] determined the number of nitrite-forming organisms in two samples of manure and found about 32,000 present per gram.

Wohltmann, Fischer and Schneider[9], and Moll[10] showed that peptone decomposition, nitrification, ammonia assimilation, and nitrogen fixation in soil were more or less strongly increased by additions of manure. Considerable increase in the peptone decomposing power of soil with applications of manure was likewise shown by Lipman[11]. Welbel[12] noted increased nitrate formation in soils receiving manure, and Wollny[13] showed a large increase in carbon dioxide production attributable, of course, to the large amount of organic material introduced and to the bacterial activities encouraged thereby.

It will not be necessary to cite the literature bearing on the subject of denitrification and nitrogen fixation as affected by manure, as the present experiments do not include work along these lines. Suffice it to say that nitrogen fixation has been shown to be increased when manure was applied to soils, and denitrification also. In the latter case, however, from the methods employed in making the tests, the results are very questionable and it is commonly believed now that unless excessive amounts of manure are applied together with a nitrate fertilizer there is little or no danger of volatilization of nitrogen by denitrification.

THE PLOTS EMPLOYED.

Five one-tenth acre plots which have been under experiment for five years were chosen for this work. The plots are located

[8]Centbl. f. Bakt., Abt. II., Bd. 26, p. 414.
[9]Journ. f. Landw., Bd. 52, 1904, p. 97-125.
[10]Beitr. Z. Biochemie d. Bodens. Diss. phil. Leipzig, 1909, p. 45-54.
[11]Rpt. New Jersey Agric. Expt. Stat. 27, 1906, p. 135.
[12]Russ. Journ. f. Expt. Landw., 4, 1903, p. 307; 6, 1905, p. 163.
[13]Landw. Vers. Stat., 36, 1899, p. 211.

on the Wisconsin drift area, the particular soil type being a Marshall loam. They are quite uniformly level. Prior to 1908 the land had been used for ordinary farming rotations and had received no special treatment. In 1908 a system of cropping according to the regular four year rotations of corn, corn, oats and clover was begun with a crop of corn. This was followed by oats in 1909 and this in turn by clover in 1910. In the fall of 1910 manure was aplied as follows:

Plot No.	Treatment.
1004—	Check
1005—	8 tons manure per acre
1006—	12 tons manure per acre
1007—	16 tons manure per acre
1008—	20 tons manure per acre

In the following year the corn crop suffered very severely from a continued drought, that on the plots receiving the manure more so than on the check plot. Evidently the manure exerted a depressing effect on the crop yields, due to the dry weather.

In 1912, however, a good season brought the yield of the second corn crop up to the normal and the beneficial effects of the manure became apparent.

The crop yields will be given later and comparisons between these and the bacteriological activities in the various plots will be made. The tests of the bacteriological activities include determinations of the number of organisms in the soils and of the ammonifying and nitrifying powers of the soils, several methods being used in these determinations.

Four samplings were made, the first on August 2, the second on August 15, the third on August 22, and the last on September 9. The results obtained at the different dates are quite satisfactory and in most cases the same differences between the various soils are evidenced.

THE METHOD OF SAMPLING.

The method of sampling employed in this work was the same as has been given in the previous reports of the study of field soils[14] and need not be described again here. The method continues to prove satisfactory and to justify the claims which have been made for it. It may be noted in this connection that samples are never drawn immediately following a rain or during a

[14]Centbl. f. Bakt., Abt. II., Bd. 35, 1912, p. 234; Research Bul. 5, Iowa Expt. Stat., 1912, p. 194.
Centbl. f. Bakt., Abt. II., Bd. 35, 1912, p. 248; Research Bul. 5, Iowa Expt. Stat., 1912, p. 203.

severe drought, as the conditions in the soil are then deemed to be too abnormal to permit of representative results. Several days should elapse before samples are drawn after a heavy rain to permit the soil to return to its normal moisture content, and to allow many of the organisms which have been washed down into the lower soil layers to return to the surface via the capillary moisture. As is known, there is considerable depression in numbers of organisms in a soil after a continued drought and species relationships are considerably altered. It is but natural, then, to expect that the differences in bacterial conditions in various plots may be very largely obscured by the death or inactivity of important species and considerable care should be exercised also in thoroughly mixing the soil in the area from which the samples are drawn in order to secure as complete uniformity as possible.

THE QUANTITATIVE DETERMINATIONS.

In the quantitative determinations two media were employed, the first being the "modified synthetic" agar used in the previous work in this series of studies of field soils, and the second an albumen agar which was devised by the writer and described in a recent publication[15]. Comparisons of these two media were given in the publication just referred to and the purpose of using them both in this experiment was to afford additional data regarding the value of the albumen agar which according to the previous results allowed of the development of much larger numbers of organisms than the modified synthetic agar.

The composition of the albumen agar is as follows:

1000 c.c. distilled water
0.5 gm. K_2HPO_4
0.2 gm. $MgSO_4$
0.1 gm. Egg Albumen
10.0 gms. dextrose
trace $Fe_2(SO_4)_3$
15.0 gms. agar

The only difference between this medium and the modified synthetic agar is that in the latter the albumen is replaced by 0.05 gm. peptone.

The usual method of making the plates was employed; that is, 100 grams of soil were shaken for five minutes with 200 c.c. of sterile water and dilutions made of 1-2,000; 1-20,000, and 1-200,000. Two 1 c.c. portions of each of these dilutions were plated, one with modified synthetic agar and the other with albumen agar. The plates were incubated for three days at room

[15]Centbl. f. Bakt., Abt. II., Bd. 38, 1913, p. 497; Research Bul. 11, Iowa Expt. Stat., 1913, p. 297.

temperature at the first date, for four days at the second, and for five days at the third and fourth dates, at the same temperature.

The results of these quantitative determinations are given in tables I and II and are calculated as usual as numbers of bacteria per gram of air-dry soil. The moisture content of the soils at the different samplings is given in table III. Glancing over this table we find very little variation in the moisture conditions in the various soils at the different dates, the largest difference at any one date being only two and one-half per cent.

The moisture conditions being so nearly uniform, the differences in bacterial numbers and activities in the various plots, as brought out by these experiments, must be attributed, therefore, to differences inherent in the soil or brought about by different treatments. Differences inherent in the soil may be very largely neglected in this case as the soil is uniform in composition and the plots are located on level, well-drained land. Hence differences in treatment must be regarded as the cause of varying bacterial activities.

RESULTS WITH MODIFIED SYNTHETIC AGAR.

Turning to table I, which gives the quantitative results with modified synthetic agar, we note that at every sampling the plots receiving eight tons of manure per acre showed greater numbers than the check plot, and the plot receiving twelve tons gave a still larger number. At two dates the maximum number of organisms seems to have been reached with this latter amount of manure and a slight depression occurred with sixteen tons, but at the other two samplings a slight increase was obtained with the larger amount of manure.

TABLE I. QUANTITATIVE DETERMINATIONS.

(Modified synthetic agar)

(Bacteria per gram of air-dry soil.)

Plot No.	Aug. 2	Aug. 15	Aug. 22	Sept. 9
1004	2,800,000	1,700,000	2,150,000	2,125,000
1005	3,143,000	2,750,000	3,031,000	2,950,000
1006	3,331,000	3,726,000	3,400,000	3,248,000
1007	3,462,000	3,713,000	3,500,000	3,151,000
1008	3,223,000	3,204,000	3,180,000	3,024,000

Any conclusion regarding the amount of manure causing the maximum increase in numbers of organisms would, therefore, hardly be justifiable. The plot receiving twenty tons of manure per acre in every case showed fewer bacteria than those receiving sixteen and twelve tons per acre, but more than that receiving eight tons per acre. If still larger amounts of manure had been employed it is a matter of doubt whether a further depression in numbers would have occurred—even perhaps below the check plot.

TABLE II. QUANTITATIVE DETERMINATIONS.
(Albumen agar)
(Bacteria per gram of air-dry soil.)

Plot No.	Aug. 2	Aug. 15	Aug. 22	Sept. 9
1004	3,153,000	6,500,000	4,550,000	2,875,000
1005	3,565,000	7,550,000	5,153,000	3,750,000
1006	3,894,000	9,496,000	6,500,000	4,315,000
1007	4,046,000	9,501,000	6,450,000	4,121,000
1008	3,741,000	8,932,000	5,693,000	3,951,000

TABLE III. MOISTURE IN SOILS.

Plot No.	Aug. 2 Per cent	Aug. 15 Per cent	Aug. 22 Per cent	Sept. 9 Per cent
1004	15.00	20.00	20.00	20.00
1005	14.75	20.00	19.50	20.00
1006	14.75	19.50	20.00	17.50
1007	14.50	19.75	20.00	17.50
1008	15.00	19.50	19.50	18.00

RESULTS WITH ALBUMEN AGAR.

When we examine table II, which gives the results obtained by the use of the albumen agar, we find that much larger numbers are shown. The difference in the incubation period of the plates at different dates which has been mentioned was hardly apparent when the modified synthetic agar was used. The maximum counts were evidently obtained in three days. With the albumen agar on the other hand the best results were secured in four to five days, which allowed time for the development of many colonies which in three days were probably too small to be visible. Greater differences in the numbers from the different plots were also shown where the longer incubation periods were employed.

Practically the same relations were observed here as were noted with the modified synthetic agar. The plot receiving eight tons of manure per acre showed larger numbers than the check plot and that receiving twelve tons per acre still larger numbers. Again the plot to which sixteen tons per acre were applied, at two dates showed slightly greater numbers and at the other two samplings slightly fewer organisms, the differences being very small. Where twenty tons of manure per acre were applied, just as with the other medium fewer organisms were shown than where twelve tons were used, but more than where eight tons were added.

The effects of the application of manure, therefore, up to a certain amount seemed to be to cause an increase in numbers of organisms developing on the modified synthetic and albumen agars. Beyond this maximum amount, which was apparently about sixteen tons per acre, there occurred a distinct decrease in numbers of organisms, fewer bacteria being found than in the soil receiving the twelve tons per acre.

The variation in the results at the different dates on the soils receiving the sixteen tons of manure make it impossible to fix the point at which the maximum effects from the manure were produced.

As has been noted already, it was not the intention in this work to examine into the causes for the results secured, consequently the reasons for the increase in organisms with applications of manure up to about sixteen tons per acre followed by a decrease when twenty tons were used may be merely suggested. The increase without doubt may be attributed primarily to the addition of large numbers of organisms, to the addition of a large amount of bacterial food, and to the presenting of more favorable physical conditions for the multiplication of the organisms present in the soil as well as those introduced.

The depression in numbers of organisms when twenty tons of manure per acre were applied may be due to one of two causes, either to the effect of the organic matter in the manure on the groups of organisms which develop on the modified synthetic and albumen agars, or to the fact that encouragement to such an extent was given to groups of bacteria which do not develop on these media that the other groups were restricted and this depression appeared in the counts while the gains in the other groups could not be determined. It would be interesting to continue this work, using larger amounts of manure and to ascertain how far this depression in numbers would go.

Comparing the results obtained in using the two media we find that greater numbers were obtained in every case with the albumen agar, the greatest difference being apparent when the incubation period was four days which was apparently the optimum incubation period for albumen agar. Furthermore, greater differences between the numbers of organisms in the different plots were given by the counts on the albumen agar than by those on the modified synthetic agar. There are, therefore, undoubtedly organisms which develop on the albumen agar which refuse to do so on the synthetic agar. The advantages which were attributed to the albumen agar in the work already cited were therefore borne out by these results. The one objection to the medium which was mentioned in the same work may be noted here as being of little importance. This objection was the cloudiness of the medium, brought about by the coagulation of the albumen when the medium was sterilized. By shaking the tubes of agar thoroughly before plating, the coagulation was broken up quite completely and gave no difficulty in the counting as the colonies were readily distinguishable from the minute particles of coagulum.

THE AMMONIFICATION EXPERIMENTS.

In the experiments to determine the ammonifying power of the soil four methods were used. These were described in a recent publication[16], and they were employed in this series to obtain additional evidence of their value. Casein, advocated in the work just referred to as the most satisfactory material to be used as a measure of the ammonifying power of soils, and albumen, which also gave evidence of some value, were employed with fresh soil as a medium. The results obtained were compared with those secured by using dried blood with fresh soil and dried blood with air-dried soil inoculated with infusions of fresh soils.

Six 100-gram portions of fresh soil obtained as already described were weighed out in tumblers and thoroughly stirred with a sterile spatula. To two of these portions 10 c.c. of a casein solution, prepared by dissolving 100 grams of casein in 1000 c.c. of water containing 70 c.c. of normal NaOH, were added. To the second two, ten c.c. of a 10% solution of albumen, prepared by dissolving sterile albumen in sterile water, were applied. To the remaining two, five grams of dried blood were added and thoroughly mixed with the soil. The moisture content of the fresh soil was determined and sterile water was added to the samples to bring them up to the optimum for the soil, 70% being considered the optimum for the dried blood. They were then covered and incubated for varying lengths of time.

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[16]Central. f. Bakt., Abt. II., Bd. 39, 1913, p. 61; Research Bul. 11, Iowa Expt. Stat., 1913, 385.

TABLE IV. THE AMMONIFICATION OF CASEIN.

Plot No.	Lab. No.	Aug. 2 Mgs. N.	Average Mgs. N.	Lab. No.	Aug. 15 Mgs. N.	Average Mgs. N.
1004 ----------------	41	38.46	------------	541	67.10	------------
	42	37.28	37.87	542	69.45	68.27
1005 ----------------	43	46.70	------------	543	72.99	------------
	44	47.09	46.89	544	74.16	73.57
1006 ----------------	45	51.40	------------	545	76.91	------------
	46	52.19	51.79	546	78.09	77.50
1007 ----------------	47	51.01	------------	547	78.09	------------
	48	52.97	51.99	548	78.87	78.48
1008 ----------------	49	48.78	------------	549	74.56	------------
	50	48.78	48.78	550	75.73	75.14

Plot No.	Lab. No.	Aug. 22 Mgs. N.	Average Mgs. N.	Lab. No.	Sept. 9 Mgs. N.	Average Mgs. N.
1004 ----------------	1041	67.10	------------	1241	52.58	------------
	1042	67.89	67.49	1242	50.62	51.60
1005 ----------------	1043	73.38	------------	1243	59.25	------------
	1044	72.20	72.79	1244	58.47	58.86
1006 ----------------	1045	78.48	------------	1245	63.32	------------
	1046	79.26	78.87	1246	66.32	66.32
1007 ----------------	1047	79.26	------------	1247	65.92	------------
	1048	79.66	79.46	1248	65.53	65.72
1008 ----------------	1049	74.16	------------	1249	60.03	------------
	1050	75.34	74.75	1250	60.82	60.42

Samples of soil from the various plots were air-dried and sieved and two 100-gram portions of each were weighed out in tumblers. Five grams of dried blood were added to each and thoroughly stirred in and 20 c.c. of infusions of fresh soils, obtained at the same time as those used in the other methods and prepared by shaking 100 grams for five minutes with 200 c.c. of sterile water, were added. The moisture content here was adjusted to the optimum with sterile water, 70% being again taken as the optimum for the dried blood. These samples were also covered and incubated at room temperature.

The distillation of all of these samples was carried on as usual, transferring to copper flasks, adding water, heavy magnesium oxide and a little paraffin, collecting the ammonia in standard acid and titrating against standard alkali.

The results obtained when using the casein are given in table IV and the summarized results in table V. At the first date of

TABLE V. THE AMMONIFICATION OF CASEIN.

Plot No.	I Mgs. N.	II Mgs. N.	III Mgs. N.	IV Mgs. N.
1004	37.87	68.27	67.49	51.60
1005	46.89	73.57	72.79	58.86
1006	51.79	77.50	78.87	66.32
1007	51.99	78.48	79.46	65.72
1008	48.78	75.14	74.75	60.42

sampling the incubation period was only three days, while at the latter three dates it was four days and consequently larger amounts of ammonia were produced in the latter cases.

The main fact to be obtained from table IV is that the duplicate determinations agree remarkably well, in most cases the agreement being as close as could be expected in a chemical method. As will be noted later, this is a great advantage for the casein method.

MANURED SOILS SHOW GREATER AMMONIFYING POWER.

Turning to table V for a summarization of the results we find that the soils receiving applications of manure all showed greater ammonifying power than the check soil. The plot receiving twelve tons of manure per acre showed a higher ammonifying power than that receiving eight tons. The soil to which sixteen tons of manure per acre were applied, except in one case when a slight decrease occurred, gave a slightly greater production of ammonia than that where twelve tons per acre were used. The application of twenty tons of manure per acre, however, decreased the ammonifying power of the soil below that given by the soil receiving twelve tons per acre, but it remained somewhat greater than that of the plot where eight tons were added. The large amount of manure in this latter case evidently depressed the activities of the ammonia producing organisms. This depression was coincident with a depression in number of organisms and hence indicates that the media employed for counting the numbers of organisms permit of the development of the ammonifying species. The largest ammonia production occurring thus in the soil receiving sixteen tons of manure per acre, it is probably a safe assumption that the maximum ammonification would occur on this particular soil type with application of manure somewhere about sixteen tons per acre.

TABLE VI. THE AMMONIFICATION OF ALBUMEN.

Plot No.	Lab. No.	I Ammonia Mgs. N.	Average Mgs. N.	Lab. No.	II Ammonia Mgs. N.	Average Mgs. N.
1004	61	17.27	-----	561	53.76	-----
	62	17.27	17.27	562	54.15	53.95
1005	63	23.15	-----	563	57.68	-----
	64	23.94	23.54	564	56.90	57.29
1006	65	26.29	-----	565	62.78	-----
	66	27.08	26.68	566	64.35	63.56
1007	67	27.08	-----	567	65.53	-----
	68	27.47	27.27	568	64.75	65.14
1008	69	23.94	-----	569	58.86	-----
	70	24.72	24.33	570	66.43	59.64

Plot No.	Lab. No.	III Ammonia Mgs. N.	Average Mgs. N.	Lab. No.	IV Ammonia Mgs. N.	Average Mgs. N.
1004	1061	57.68	-----	1261	54.15	-----
	1062	54.15	55.91	1262	52.97	53.56
1005	1063	62.00	-----	1263	57.68	-----
	1064	62.00	62.00	1264	58.47	58.07
1006	1065	67.89	-----	1265	70.24	-----
	1066	69.85	68.87	1 66	68.67	69.45
1007	1067	67.89	-----	1267	69.45	-----
	1067	70.24	69.06	1268	71.02	70.23
1008	1069	64.35	-----	1269	67.49	-----
	1070	62.78	63.56	1270	69.06	68.28

Table VI contains the results of using albumen as the measure of the ammonia producing power of the soil. The samples at the first date were incubated only four days while at the later samplings six days was the length of incubation. Hence the ammonia production at the first date was very much less than at the later samplings and the differences between the various soils were smaller. Six days seemed to be the optimum period of incubation. The duplicate determinations agreed quite closely, in only one or two cases were there discrepancies of any size.

In table VII, which gives the summarized results with albumen, it may be noted that the increased ammonifying power produced in the soil by addition of manure was again evidenced. Again the application of twelve tons per acre caused a larger

TABLE VII. THE AMMONIFICATION OF ALBUMEN.

Plot No.	I Ammonia Mgs. N.	II Ammonia Mgs. N.	III Ammonia Mgs. N.	IV Ammonia Mgs. N.
1004	17.27	53.95	55.91	53.56
1005	23.54	57.29	62.00	58.07
1006	26.68	63.56	68.87	69.45
1007	27.27	65.14	69.06	70.23
1008	24.33	59.64	63.56	68.28

increase than eight tons. Sixteen tons in every case here caused a slightly greater production of ammonia than twelve tons. The conclusion reached from the results obtained with casein are therefore confirmed; that is, it seems that manure applied to soil at the rate of about sixteen tons per acre caused the maximum ammonia production.

The larger amount of manure, twenty tons just as in the previous case, reduced the ammonia production below that from the soils receiving twelve and sixteen tons but it still remained larger than that from the soil receiving eight tons per acre.

These results again coincided very largely with the results of the quantitative determinations and further evidence was thus supplied that the media employed in the latter determinations permit of the development of the ammonia producing organisms to a very large extent.

The results obtained by the use of dried blood in fresh soil are given in table VIII and the summarized results in table IX. These samples were incubated for five days, which has been found to be the optimum period for the ammonification of dried blood.

The duplicate determinations here, as is always the case when dried blood is employed, did not agree very closely. Such a large amount of ammonia is produced, it is so difficult to mix the dried blood thoroughly with the soil, and the difficulty in distilling because of foaming is so great that close agreement of duplicates is always impossible. Examining the summarized results in table IX we find that the effects of the manure on the ammonia production were quite pronounced.

The soil receiving twelve tons of manure per acre showed greater ammonifying power than that receiving eight tons, which in turn gave a greater ammonia production than the check

TABLE VIII. THE AMMONIFICATION OF DRIED BLOOD.
(Fresh Soil)

Plot No.	Lab. No.	I Ammonia Mgs. N.	Average Mgs. N.	Lab. No.	II Ammonia Mgs. N.	Average Mgs. N.
1004	21	68.28		521	85.94	
	22	65.53	66.90	522	82.01	83.97
1005	23	86.72		523	94.18	
	24	82.80	84.76	524	90.25	92.21
1006	25	90.64		525	102.81	
	26	82.01	86.32	526	109.87	106.34
1007	27	100.06		527	107.91	
	28	95.75	97.90	528	111.05	109.47
1008	29	87.90		529	101.24	
	30	85.54	86.72	530	90.25	95.74

Plot No.	Lab. No.	III Ammonia Mgs. N.	Average Mgs. N.	Lab. No.	IV Ammonia Mgs. N.	Average Mgs. N.
1004	1021	74.95		1221	67.10	
	1022	72.20	73.57	1222	66.32	66.71
1005	1023	85.94		1223	71.42	
	1024	82.01	83.97	1224	69.85	70.63
1006	1025	96.53		1225	84.76	
	1026	101.24	98.88	1226	86.32	85.54
1007	1027	95.35		1227	86.32	
	1028	102.42	98.88	1228	83.58	84.95
1008	1029	89.07		1229	78.48	
	1030	85.94	87.50	1230	75.34	76.91

TABLE IX. THE AMMONIFICATION OF DRIED BLOOD.
(Fresh Soil)

Plot No.	I Ammonia Mgs. N.	II Ammonia Mgs. N.	III Ammonia Mgs. N.	IV Ammonia Mgs. N.
1004	66.90	83.97	73.57	66.71
1005	84.76	92.21	83.97	70.63
1006	86.32	106.34	98.88	85.54
1007	97.90	109.47	98.88	84.95
1008	86.72	95.74	87.50	76.91

TABLE X. THE AMMONIFICATION OF DRIED BLOOD.
(Air-dry Soil)

Plot No.	Lab. No.	I Ammonia Mgs. N.	Average Mgs. N.	Lab. No.	II Ammonia Mgs. N.	Average Mgs. N.
1004	1	72.59		501	113.80	
	2	88.29	80.44	502	109.87	111.83
1005	3	96.14		503	113.80	
	4	93.39	94.76	504	120.86	117.33
1006	5	102.02		505	127.92	
	6	98.10	100.06	506	134.59	131.25
1007	7	104.38		507	153.43	
	8	97.32	100.85	508	120.86	137.14
1008	9	97.32		509	118.11	
	10	94.18	95.75	510	139.69	128.90

Plot No.	Lab. No.	III Ammonia Mgs. N.	Average Mgs. N.	Lab. No.	IV Ammonia Mgs. N.	Average Mgs. N.
1004	1001	113.01		1201	114.19	
	1002	99.67	106.34	1202	91.43	102.81
1005	1003	105.16		1203	114.97	
	1004	113.79	109.47	1204	119.29	117.13
1006	1005	129.10		1205	133.81	
	1006	115.37	122.23	1206	122.04	127.92
1007	1007	129.10		1207	137.34	
	1008	118.90	124.00	1208	128.71	133.02
1008	1009	116.94		1209	121.64	
	1010	110.66	113.80	1210	123.61	122.62

TABLE XI. THE AMMONIFICATION OF DRIED BLOOD.
(Air-dry Soil)

Plot No.	I Ammonia Mgs. N.	II Ammonia Mgs. N.	III Ammonia Mgs. N.	IV Ammonia Mgs. N.
1004	80.44	111.83	106.34	102.81
1005	94.76	117.33	109.47	117.13
1006	100.06	131.25	122.23	127.92
1007	100.85	137.14	124.00	133.02
1008	95.75	128.90	133.80	122.62

soil. Where sixteen tons per acre were applied, at two dates there was shown a still greater ammonifying power, and at the other two samplings practically identical figures were obtained as where twelve tons were used. We may conclude again, therefore, that applications of about sixteen tons of manure per acre bring the soil to its maximum ammonifying power. The twenty ton application, however, just as was the case when the casein and albumen were used, depressed the ammonifying power of the soil below that of the soils receiving twelve and sixteen tons per acre, but hardly down to that of the soil to which eight tons were applied.

The results secured by this method confirm very largely those secured in the other cases and therefore the same agreement with the results of the quantitative determinations is to be noted.

An examination of table X for the results secured by using air-dried soil wih dried blood and inoculations of fresh infusions shows that the agreement between the duplicate determinations was very poor although the averages give about the same difference between the various soils as were shown by the other methods. These differences in duplicates by this method are unavoidable for the reasons which have been discussed. These samples were all incubated for six days, which has been shown to be the optimum period for dried blood in air-dry soil.

In table XI the average results show the ammonifying power of the different soils by this method. The application of eight tons of manure per acre caused a decided increase in the ammonifying power of the soil, twelve tons, a greater increase, and sixteen tons a still further increase, which however, at two dates was very slight. The twenty tons again depressed the ammonifying power of the soil below that shown where twelve and sixteen tons were employed but not below that where eight tons were applied. These results coincided very satisfactorily with those secured by the other methods.

Again, the effect of applications of manure in increasing the ammonifying power of the soil is clearly shown, the maximum amount of ammonia being produced when about sixteen tons of manure per acre were used. Beyond sixteen tons there occurred a depression in ammonifying power similar to that shown by the other methods. Again the results corresponded closely with the results of the quantitative determinations.

FACTS THAT STAND OUT IN AMMONIFICATION RESULTS.

Considering these ammonification results as a whole several facts should be emphasized. In the first place the effect of applications of barnyard manure on the ammonifying power of the soil was quite definitely shown. The increases secured were too great to be accounted for merely on the basis of the plant food actually added in the manure. Of course the physical factors were of some importance but in this case greater effects have been noted than could be ascribed to them.

It seems, therefore, from these results that the effects of manure may be very largely due to the influence on the bacterial activities. Whether this influence is due to the chemical, physical, or bacteriological composition of the manure or to its physiological effect on bacteria in stimulating their growth cannot be stated, but it is undoubtedly the case that the effect on crop growth may be traced directly to the effect on certain bacterial activities. The ammonifying power of a soil, representing as it does the production of available nitrogenous material for plant growth, must therefore be linked up closely with crop production. That is, unless conditions are exceedingly abnormal in a soil, it may be assumed that the activities of the ammonifying organisms are indicative of the crop-producing power of the soil.

In the earlier work in this series of studies of field soils, the actual crops produced and the ammonifying power of the soils as measured in the laboratory have been found to be very closely correlated. The crop yields from the plots used in these experiments will be given later and their correspondence with these ammonification results noted.

NUMBERS OF BACTERIA AND AMMONIFICATION CLOSELY RELATED.

In the second place evidence is supplied that numbers of bacteria and ammonification are very closely related. This is in accord with the results already secured and is of much interest from the fertility standpoint. As has just been stated, we know that its ammonifying power may be a very close measure of the fertility or crop-producing power of a soil. The numbers of organisms found by the use of the albumen and modified synthetic agars varied in the different soils exactly as the ammonifying power of the soils varied and hence it may be concluded that the estimate of the numbers of bacteria in the soils included at least the major portion of the ammonifying species, and furthermore, that a definite relation between numbers and crop production may be traced.

Finally, the fact that too heavy applications of manure may be made to a soil was quite definitely shown. When more than sixteen tons of manure per acre were used a depression in the ammonifying power of the soil occurred corresponding to a decrease in numbers. It is evident therefore that too much manure may be positively injurious. It will be shown later that the crop yields were also reduced by the large amount of manure.

FRESH SOIL-CASEIN METHOD PROVES BEST.

Comparing the results secured by the various methods for the study of ammonification, previous conclusions were borne out by the figures at hand.

The most satisfactory method was again found to be the fresh soil-casein method. The differences were more pronounced when it was used, the duplicates agreed the best and the least difficulty in distilling was encountered. When albumen was used the main difficulty appeared in the preparation of a sterile solution. This was so difficult that in view of the fact that the albumen possesses no advantage over the casein, the method cannot be recommended. When the fresh soil-dried blood-method was used, the amounts of ammonia produced were so large and so much carbon dioxide was formed that there was great difficulty in distilling. This fact, together with the difficulty in mixing the dried blood thoroughly with the soil, may be held accountable for the poor agreement of duplicates. When the dried blood was used with air-dry soil the same difficulties were experienced, and an additional objection to the method lies in the fact that air-dry soil is not sterile and soil infusions may not be absolutely representative, and hence the results secured by the method cannot be depended upon as representing field conditions.

In short, the casein method according to these results, possesses many advantages over the other methods and previous claims made for it are entirely supported.

THE NITRIFICATION EXPERIMENTS.

The nitrification experiments were carried out by using ammonium sulfate with fresh soil and with air-dry soil. The use of fresh soil has seemed by far the most rational method in examining soils, and it was desired to compare its results with those obtained with air-dry soil.

One hundred gram quantities of fresh soils were weighed off in tumblers, stirred thoroughly and one c.c. portions of a 10% solution of ammonium sulfate added.

The moisture content of the soils was ascertained and then brought up to the optimum with sterile water. The tumblers were covered and incubated for four weeks, the water content being kept constant by making up to weight with sterile water every week.

At the same time 100-gram quantities of air-dry sieved soil from the various plots were weighed off, one c.c. portions of a 10% ammonium sulfate solution added, and twenty c.c. of five minute infusions of fresh samples of the corresponding soils

TABLE XII. THE NITRIFICATION OF $(NH_4)_2\ SO_4$.
(Fresh Soil)

Plot No.	Lab. No.	I Aug. 2 Mgs. N.	Average Mgs. N.	Lab. No.	II Aug. 15 Mgs. N.	Average Mgs. N.
1004	141	5.000		641	11.000	
	142	6.152	5.576	642	10.892	10.946
1005	143	7.156		643	12.500	
	144	7.362	7.259	644	12.667	12.583
1006	145	8.379		645	16.665	
	146	8.561	8.470	646	16.802	16.733
1007	147	10.164		647	18.500	
	148	10.400	10.282	648	18.888	18.694
1008	149	8.052		649	16.000	
	150	8.199	8.125	650	16.328	16.164

Plot No.	Lab. No.	III Aug. 22 Mgs. N.	Average Mgs. N.	Lab. No.	IV Sept. 9 Mgs. N.	Average Mgs. N.
1004	1141	10.000		1341	9.282	
	1142	10.567	10.283	1342	9.000	9.141
1005	1143	12.359		1343	10.000	
	1144	12.728	12.543	1344	10.000	10.000
1006	1145	14.285		1345	12.500	
	1146	14.000	14.142	1346	12.896	12.698
1007	1147	15.282		1347	12.800	
	1148	16.000	15.641	1348	13.222	13.011
1008	1149	13.000		1349	10.228	
	1150	12.899	12.949	1350	10.829	10.528

TABLE XIII. THE NITRIFICATION OF $(NH_4)_2SO_4$.
(Fresh Soil)

Plot No.	I Mgs. N.	II Mgs. N.	III Mgs. N.	IV Mgs. N.
1004	5.576	10.946	10.283	9.141
1005	7.259	12.583	12.543	10.000
1006	8.470	16.733	14.142	12.698
1007	10.282	18.694	15.641	13.011
1008	8.125	16.164	12.949	10.528

were introduced. The moisture content was adjusted and maintained during the incubation period of four weeks as in the other method.

The results of the tests with fresh soils are given in table XII. The duplicate determinations agreed very satisfactorily as shown in table XIII which gives the summarized results.

In every case the manure increased the nitrifying power of the soil up to sixteen tons per acre. Beyond that, however, a reduction occurred, twenty tons depressing the nitrate production below that shown by the soil receiving twelve tons per acre. The results were quite in agreement with those secured in the quantitative determinations and ammonification experiments except that in those cases the maximum amount of manure was not so definitely shown. Here the greatest nitrifying power in every case appeared in the soil receiving sixteen tons of manure per acre.

The results by the use of air-dry soil are given in table XIV and here, too, the duplicates were quite satisfactory. In table XV the summarized results of the tests appear and upon examination it is found that differences in the nitrifying power of the soil were obtained here similar to those secured by the use of fresh soil. Again the application of manure up to sixteen tons per acre increased the nitrifying power of the soils, twelve tons giving a larger nitrate production than eight tons, and sixteen tons more than twelve. There was one exception to this increase, however, at the last date of sampling a slightly smaller nitrate production was noted in the plot receiving sixteen tons of manure than in that to which twelve tons were applied. The difference was so slight that it would be negligible were it not for the fact that the same slight decrease occurred at this date in the numbers of organisms and in the ammonifying power measured by casein or by dried blood in fresh soil, in the soil receiving sixteen tons over that to which twelve tons per acre were added. It might seem therefore that at that particular date, or in the particular sample some peculiarity or unavoidable contamination altered the results from their common trend at the other dates of sampling.

Here again the application of twenty tons of manure depressed the nitrifying power of the soil below that shown when only twelve tons were applied.

The results also check quite satisfactorily the results of the quantitative determinations and the ammonification experiments.

As a whole the nitrification experiments bring out the same facts as were obtained by the ammonification and quantitative studies. The increases in numbers and ammonifying power were accompanied by increases in nitrifying power. Depressions in numbers and ammonia production were likewise fol-

TABLE XIV. THE NITRIFICATION OF $(NH_4)_2SO_4$.
(Air-dry Soil)

Plot No.	Lab. No.	I Nitrate Mgs. N.	Average Mgs. N.	Lab. No.	II Nitrate Mgs. N.	Average Mgs. N.
1004	121	8.335		621	14.689	
	122	8.679	8.507	622	14.900	14.794
1005	123	9.260		623	15.350	
	124	9.392	9.326	624	15.557	15.453
1006	125	10.000		625	17.892	
	126	10.000	10.000	626	17.528	17.710
1007	127	11.905		627	18.500	
	128	11.405	11.655	628	18.925	18.712
1008	129	10.000		629	16.667	
	130	10.128	10.064	630	16.725	16.696

Plot No.	Lab. No.	III Nitrate Mgs. N.	Average Mgs. N.	Lab. No.	IV Nitrate Mgs. N.	Average Mgs. N.
1004	1121	12.500		1321	9.422	
	1122	12.500	12.500	1322	9.000	9.211
1005	1123	13.800		1323	10.000	
	1124	13.586	13.693	1324	10.525	10.262
1006	1125	14.285		1325	12.500	
	1126	14.500	14.392	1326	12.687	12.593
1007	1127	16.666		1327	12.000	
	1128	16.137	16.401	1328	12.892	12.446
1008	1129	14.500		1329	10.000	
	1130	14.825	14.662	1330	10.889	10.444

TABLE XV. THE NITRIFICATION OF $(NH_4)_2SO_4$.
(Air-dry Soil)

Plot No.	I Nitrate Mgs. N.	II Nitrate Mgs. N.	III Nitrate Mgs. N.	IV Nitrate Mgs. N.
1004	8.507	14.794	12.500	9.211
1005	9.326	15.453	13.693	10.262
1006	10.000	17.710	14.392	12.593
1007	11.655	18.712	16.401	12.446
1008	10.064	16.696	14.662	10.444

lowed by decreases in nitrate production. The amount of manure bringing about maximum bacterial development among ammonifying species also caused maximum multiplication and activity of the nitrifying species. Previous results which showed that ammonification and nitrification proceeded parallel were confirmed by the work at hand.

The reasons for the increases and decreases in ammonifying and nitrifying power by the use of manure may be assumed to be the same as were mentioned in the discussion of the quantitative results. That is, the increases may be due either to additions of ammonifying and nitrifying organisms, or to the abundant bacterial food supply added which encourages those organisms already present in the soil to greater development. The decrease when too much manure was used may be due to action of the organic matter in the manure on the ammonifying and nitrifying organisms or possibly to the introduction of competing organisms to such an extent that the prominent ammonifiers and the nitrifiers were hindered in their development.

Comparing the nitrifying power of the soils as shown by the use of fresh and air-dry soil, much greater differences were found between the different soils when fresh soil was used. The greater differences are apparently due to the fact that the nitrifying power of the check soil was lower when tested by the fresh soil than when air-dry soil was used. Just why this should be so is not apparent, but there can be no doubt but that the fresh soil approaches more closely the natural conditions and hence is more nearly representative of what is occurring in the field.

THE CROP YIELDS.

Table XVI gives the yields of corn for 1912 from the plots used in these experiments. The figures given show that eight tons of manure increased the yield from 50.50 bushels to 77.62 bushels; twelve tons increased this to 86.00 bushels and sixteen tons gave a slight gain to 87.00 bushels. Twenty tons, however, caused a depression in yield to 81.00 bushels, which was below that from the plot receiving twelve tons.

Comparing these results with the bacteriological data almost absolute agreement is found. Eight tons of manure increased the number of organisms, it increased the ammonifying and nitrifying power of the soils over the check soil and likewise increased the crop yield. Twelve tons gave a further increase in crop, corresponding to an increase in numbers of bacteria, and in ammonifying and nitrifying powers. Sixteen tons showed a very slight gain in crop, and with a few exceptions, where the

TABLE XVI. THE CROP YIELDS.
(Treatment and Yield per acre.)

Plot No.	Treatment	Corn (1912)
1004	Check	50.50 bu.
1005	8 T. Manure	77.62 bu.
1006	12 T. Manure	86.00 bu.
1007	16 T. Manure	87.00 bu.
1008	20 T. Manure	81.00 bu.

differences were very small, a slight gain in numbers of bacteria and in ammonifying and nitrifying power occurred. Finally twenty tons of manure per acre depressed the crop yield below that produced with twelve tons and the numbers of bacteria, ammonifying and nitrifying powers of the soil were depressed to just that extent.

These results therefore give additional confirmation to the conclusion drawn in previous study of field soils that bacterial activities and crop production were very closely related. Furthermore, the suggestion that the determination of bacterial activities may be a means of determining the fertility or crop-producing power of a soil or at least the relative fertility of several soils is worthy of consideration in the light of the present results.

It may be suggested that the depression in crop yields by the application of twenty tons of manure per acre is due to denitrification and that the decrease in numbers, of bacteria, etc., is due to the introduction of denitrifying bacteria. This theory, however, will not account for the results secured, as tests of the denitrifying power of the soils were carried out both by the use of the Giltay solution and by the use of soil cultures, and in no case was any denitrification observed. The nitrates used in the media very rapidly disappeared but upon analysis the entire amount has been found to have been changed into protein, and no loss whatever occurred.

It may be stated here that it is probably the case that many instances of reported denitrification have been made on the basis of the disappearance of nitrates, and if chemical analyses had been made no losses of nitrogen would have been found.

At any rate the depression in crop yield which followed application of a large amount of manure cannot be attributed to denitrification in this case but to some other influence, possibly as has been suggested, physiological, and the danger of denitrification in field soils which has been emphasized in some quarters may be regarded as open to question.